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BACTERIAL FLORA. THE ROLE OF ENVIRONMENTAL FACTORS*

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ABSTRACT

It was concluded from this study that bacterial skin populations in certain areas of the body, namely the back, the groin, and the hands, increase with increasing temperature and humidity. Neither high temperature alone nor high humidity alone will provoke a similar increase under the experimental conditions described. Horne (1) observed that a minimum temperature *and* humidity appear to be important in the seasonal variation of certain infectious skin diseases, and this study provides some experimental basis for his observation.

Seasonal variations in the incidence of superficial cutaneous infections have been observed, showing more frequent occurrence during hot, humid seasons (1, 2). Certain cutaneous pathogens are found to have temperature optima slightly lower than 37° C, corresponding to the skin surface temperature, 30–32° C, rather than core temperature. Regardless of these slight differences in optima, it is well known that bacteria proliferate more rapidly in a warm, moist environment. Several investigators (1, 2) have shown beyond doubt that water is critically important in regulating the total number of organisms on the skin. The factor of environmental heat has in most clinical reports been synonymous with high humidity (3), and in some investigative studies has been an integral factor in producing the local hydration (5).

Although numerous factors undoubtedly contribute to the initiation of infection, an increase in bacterial population on the skin might supply the necessary infectious dose. Studies in which the environmental effects of temperature and humidity could be controlled separately might provide useful information on the factors which regulate the cutaneous microflora. The problem has been studied by examining the changes in bacterial populations on the skin when high temperatures and humidities are supplied both separately and together in a climate-controlled chamber.

MATERIALS AND METHODS

Climate conditions. The following three different conditions of temperature and relative humidity were studied: 90 degrees F, 95% relative humidity (Group A); 70 degrees F, 95% relative humidity (Group B); 90 degrees F, and 20% relative humidity (Group C).

Subjects. Three groups of ten men between the ages of 20 and 40 were used as subjects in the three different controlled climates. Subjects wore street clothes and remained in the controlled environment for 60 hours, during which time only hand washing was permitted. No work was performed in the chamber. After leaving the chamber, subjects were provided with soap containing no antibacterial agents for bathing during the four-day follow-up period in the natural environment.

Sampling procedure. Bacterial cultures were taken from the interscapular area of the back, the groin at a point two-thirds the distance from the anterior superior iliac spine to the pubic tubercle, palms and soles. They were cultured upon entering the chamber, after 15 hours in the experimental condition and prior to leaving at 60 hours. Follow-up cultures were taken from the same areas after 24 and 96 hours in the natural environment. Duplicate cultures were taken on the right and left sides of the body by swabbing 16 sq cm areas defined by sterile templates. Calcium alginate swabs moistened in saline buffered to pH 7.2 with 0.1% Triton-X 100 were used to swab each area twice. Swabs were returned to 3 ml of the collecting medium and shaken 10 mintues on a wrist action shaker.

CULTURE METHODS

Quantitation of bacterial population. Samples were suitably diluted and plated in duplicate on Casman's sheep blood agar using the drop plate method (6). One set of cultures was incubated aerobically at 31° C. for four days. The duplicate set was incubated anaerobically using a Brewer's anaerobic jar at 30° C. for 3 weeks. To eliminate facultative anaerobic organisms from the total anaerobic count, the replicate plate method was used (7). Replicate plates were incubated aerobically and compared with the original anaerobic plate. All organisms suspected of being anaerobic by the replicate plate technique were tested separately. From this the anaerobic count was established. The number of bacteria per square centi-

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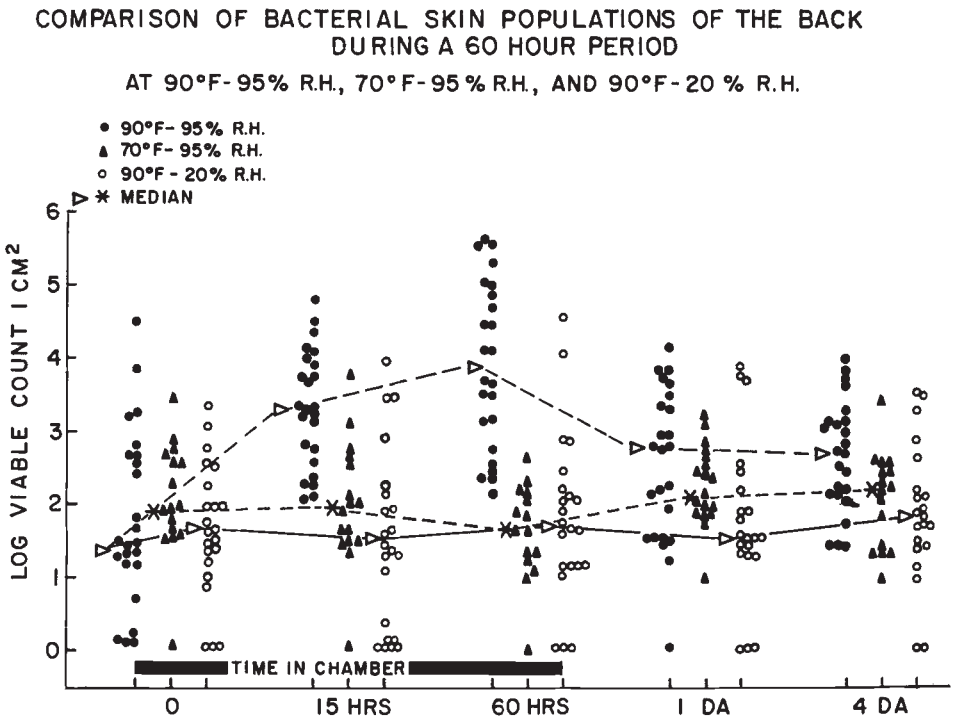

FIG. 1. Comparison of bacterial skin populations of the back during a 60 hour period

meter of skin was calculated and transformed to the log value.

Qualitative changes in bacterial populations. Each sample was cultured on Casman's sheep blood agar and other selective and differential media were used when necessary for routine identification. *Staphylococcus aureus* was identified by the coagulase and mannitol reaction. *Corynebacteria minutissimum* was identified by fluorescence on tissue culture medium No. 199 containing 20% fetal bovine serum. Other bacteria found were categorized as follows: *Staphylococcus epidermidis,* micrococci, aerobic diphtheroids, anaerobic diphtheroids, gram negative rods, and gram negative cocci. Changes in the types of bacteria found on a subject were recorded throughout the experimental period. No particular attempt was made to enumerate lipophilic diphtheroids separately (8). These were cultured as pin point colonies on Casman's sheepblood agar and were part of the total population calculated.

RESULTS

Quantitative. A comparison of bacterial populations of the skin of each group of subjects before, during and after 60 hours of the three climates are shown in a series of scattergrams (Figs. 1, 2, 3, 4) demonstrating the distribution of bacterial populations on the four different body areas under the three different conditions of temperature and relative humidity (90° F—95% RH, Group A; 70° F—95% RH, Group B; 90° F—20% RH, Group C). The median values are shown on the figures. Figure 1 shows the changes that occur in bacterial populations of the back at the three climates. Under the conditions of 90° F—95% RH, a rise is noted from the median value of log 1.40 to log 3.30 at 15 hours and to log 3.90 at 60 hours, with a decrease following one day in the natural environment to log 2.75 and no further decrease after four days in the natural environment. The medians for Groups B and C where either the temperature or humidity was reduced can be seen to change very little from the initial values. The counts were found to be distributed over a wide range throughout the experimental period. Upon entering the climate controlled chamber, counts ranged between log 0.10 and log 4.5 in Group A, log 0.10 and log 3.45 in Group B, and log 0.05 and log 3.35 in Group C. After 15 hours the counts of Group A ranged between log 2.05 and log 4.80 as opposed to Group B with a range from log 0.10 to log 3.80

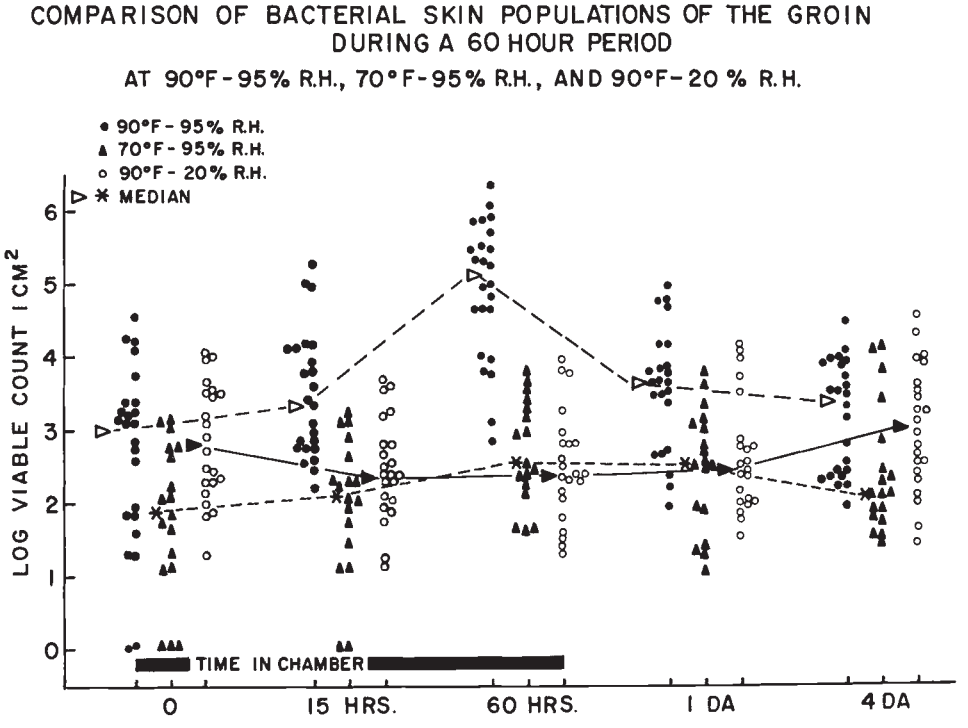

FIG. 2. Comparison of bacterial skin populations of the groin during a 60 hour period

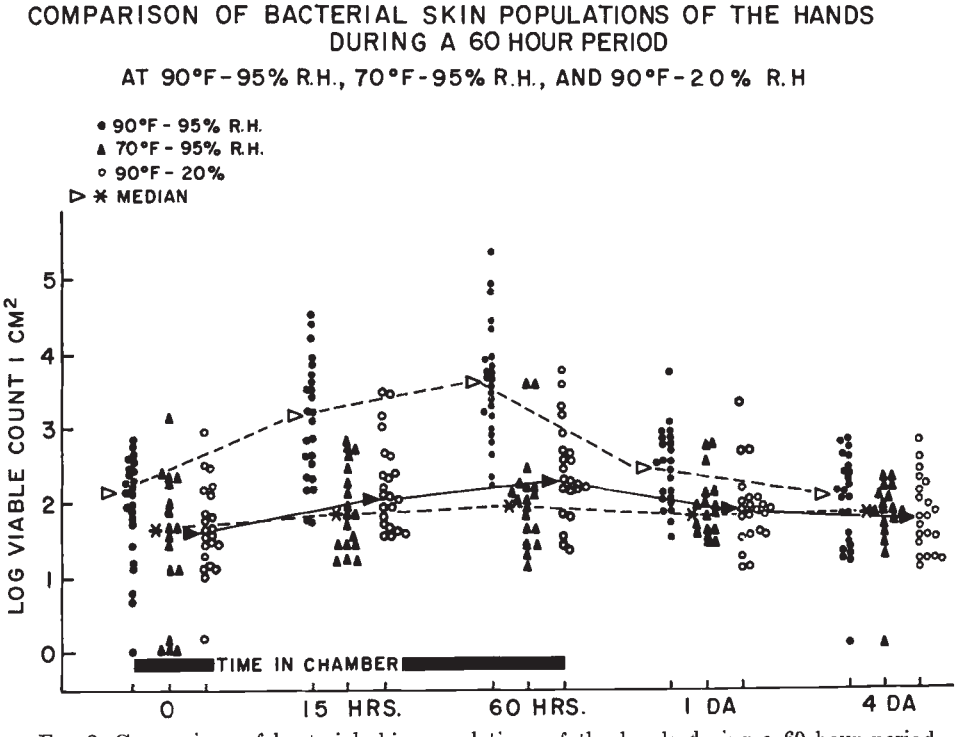

FIG. 3. Comparison of bacterial skin populations of the hands during a 60 hour period

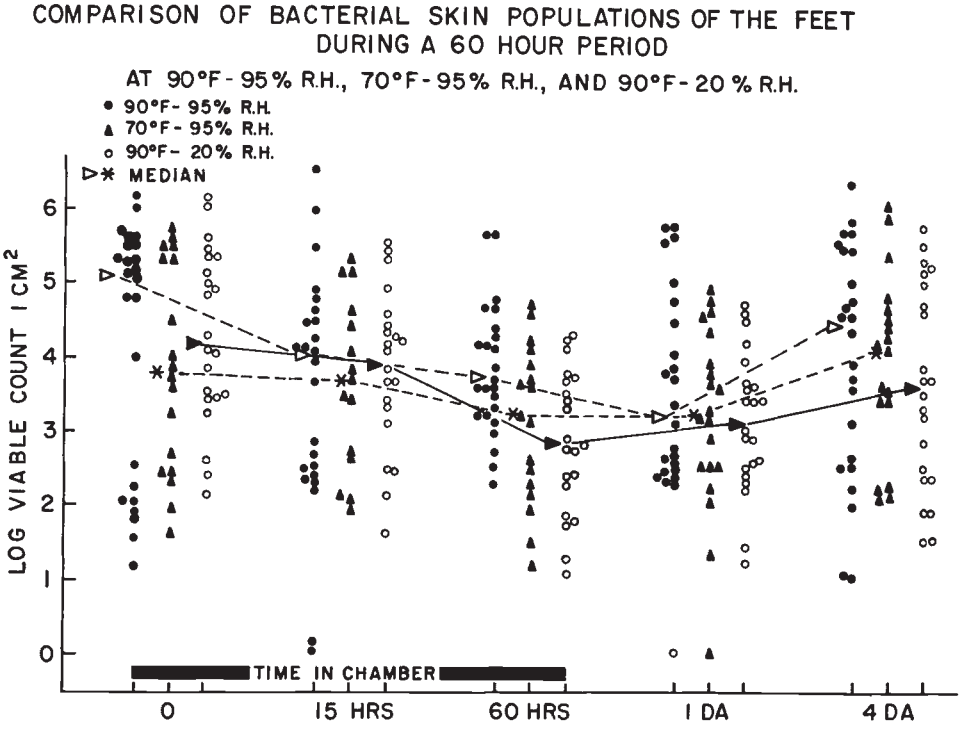

FIG. 4. Comparison of bacterial skin populations of the feet during a 60 hour period

and Group C with a range from log 0.05 to log 3.95. After 60 hours at a temperature of 90° F and 95% relative humidity the lowest count was log 2.15 and the highest count was log 5.65. In Group B where the temperature was low but the humidity was 95%, the count ranged between log 0.05 and log 2.65. Group C which had a low relative humidity and high temperature showed more variation with values ranging between log 0.05 and log 4.55 During the follow-up period in the natural environment, the bacterial population of Group A gradually decreased with final values after four days between log 1.45 and log 4.00. This approximates the other two groups which ranged from log 1.0 and log 3.45, and log 0.05 to log 3.55, respectively.

Comparable results can be seen for the groin in Figure 2 and the hands in Figure 3, despite the fact that hand washing was permitted during the experiment. The feet, however, failed to show similar increases in bacterial populations at 90° F, 95% RH, as shown in Figure 4. The counts for the feet are distributed over a wider range than the other body areas. With the exception of the initial high count at 90° F—95% RH, the differences between the median values of the three groups are slight.

The mean values, in log numbers, for the bacterial population of each body area are listed in Table I. The bacterial populations of the back, the groin and the hands increased rapidly under the conditions of 90° F—95% RH. This rise was highly significant ($p < 0.001$).

The bacterial populations of the feet did not rise under any of the experimental conditions. There was instead a significant decline in the populations at 70° F—95% RH ($p < 0.05$) and at 90° F—20% RH ($p < 0.001$). This decline during the sixty hours under the experimental conditions was followed by a significant ($p < 0.05$) rise in both groups after leaving the climate chamber. There was no significant change in bacterial counts from the feet of the group studied at 90° F—95% RH.

The anaerobic counts paralleled the aerobic counts, i.e., in the majority of subjects there was no change (under the experimental conditions) of 90° F—20% RH or 70° F—95% RH. At the 90° F—95% RH, the majority of the anaerobic counts showed an increase of log 1.5 or greater while in the chamber, with a de-

TABLE I

Mean values, in log numbers, for bacterial populations of each body area

	0 hours	15 hours	60 hours	1 day	4 days
Back					
90° F.—95% RH	2.09 ± 0.95	3.27 ± 0.88	4.07 ± 1.16	2.61 ± 1.06	2.77 ± 0.83
70° F.—95% RH	2.01 ± 1.07	1.70 ± 0.97	1.40 ± 0.80	1.96 ± 0.88	1.94 ± 0.92
95° F.—20% RH	1.66 ± 0.94	1.46 ± 1.23	1.81 ± 1.17	1.70 ± 1.13	1.82 ± 1.01
Groin					
90° F.—95% RH	2.65 ± 1.19	3.51 ± 0.84	4.95 ± 0.94	3.56 ± 0.83	3.25 ± 0.72
70° F.—95% RH	2.16 ± 1.22	2.23 ± 1.09	2.61 ± 0.68	2.44 ± 0.75	2.52 ± 0.88
95° F.—20% RH	2.85 ± 0.79	2.49 ± 0.70	2.54 ± 0.80	2.64 ± 0.78	3.05 ± 0.89
Hands					
90° F.—95% RH	1.94 ± 0.71	3.14 ± 0.79	3.78 ± 0.85	2.44 ± 0.50	1.97 ± 0.68
70° F.—95% RH	1.62 ± 0.96	1.93 ± 0.52	2.26 ± 0.79	1.81 ± 0.62	1.81 ± 0.72
95° F.—20% RH	1.65 ± 0.62	2.24 ± 0.61	2.42 ± 0.66	1.89 ± 0.52	1.71 ± 0.62
Feet					
90° F.—95% RH	4.19 ± 1.71	3.57 ± 1.64	3.83 ± 0.88	3.49 ± 1.39	4.06 ± 1.57
70° F.—95% RH	4.16 ± 1.47	3.59 ± 1.65	3.22 ± 1.00	3.41 ± 1.25	4.17 ± 1.35
95° F.—20% RH	4.30 ± 1.17	3.88 ± 1.05	2.77 ± 1.14	3.19 ± 0.96	3.65 ± 1.39

cline after returning to the natural environment.

Qualitative. In general, there was no qualitative change in the bacterial flora of the subjects. No individual experienced a remarkable or lasting change in his flora during the experiment. *Staphylococcus aureus* was isolated from five subjects in Group A; one in Group B; and two in Group C. It was never a predominant organism. *Corynebacterium minutissimum* was found on 21 subjects (62%). Gram negative organisms (*Proteus mirabilis, Aerobacter aerogenes*) were found in significant (but not predominant) numbers in one individual at 15 hours and 60 hours at 90° F—95% RH; however, they were not found after leaving the chamber.

DISCUSSION

To our knowledge, this is the first study in which the environmental effects of temperature and humidity have been controlled separately to determine what effect each might have on the cutaneous microflora.

The climates were chosen so that the skin would be warm and moist, cool and moist, and warm and dry. The warm and moist needs no further explanation. At 70° F—95% RH, the subjects were chilly and all wore long trousers and light sweaters for comfort. The dampness of the bedding and their clothing was notable. At 90° F—20 %RH, the subjects complained bitterly about the dryness, particularly of the lips and mucous membranes. All developed symptoms of mild xerosis. Visible sweating occurred in two subjects only.

The bacterial skin populations of the back, groin and hands increase with increasing temperature and humidity. It appears that neither high environmental temperature (90° F) nor high relative humidity (95%) alone will cause a similar increase under the experimental conditions described.

The results obtained from the feet are not surprising. The initial group studied was that at 90° F—95% RH, and because of profuse sweating they were allowed to wear thong-type shower shoes. It would appear there was no real difference between the "private climate" afforded by their street shoes and the experimental climate and therefore no change in bacterial counts occurred. On the other hand, there was a large difference between the "private climates" of the feet of the remaining two groups and their respective experimental climates. The conditions of 70° F—95% RH and 90° F—20% RH were less than optimum for bacterial growth and the counts fell during the period in the chamber. With return of the "private climate" associated with wearing street shoes, the bacterial populations rose again despite the fact that most of the subjects claimed to bathe daily.

The swabbing technique utilized in this study does not remove bacteria as completely as the cup method described by Williamson and Kligman (9), but in our hands is statistically as accurate and reproducible (10). The addition of Triton-X 100 to the collecting medium has been shown to break up bacterial aggregates, thereby providing bacterial counts rather than colony counts.

No superficial cutaneous infection or other untoward effect was noted during the experiment despite the significant increase in cutaneous flora during the 90° F—95% RH experiment.

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